

Figure S1. SOX9 ChIP and ChIP-Seq profiling of known SOX9 target genes in VCaP cells . A. SOX9 ChIP assay for a Col2a1 gene binding site using two independent SOX9 antibodies (Ab1 and Ab2). **B.** Known SOX9 targets in human, mouse and chicken. Adapted from transcription factor encyclopedia by Ralf Kist. <http://www.cisreg.ca/cgi-bin/tfe/articles.pl?tfid=495&tab=targets>. **C.** Binding profiles of known SOX9 targets by SOX9 ChIP-Seq. Chn: chicken. **D.** Enriched motifs of transcriptional factors within 600 bp of the summits of SOX9 binding peaks.

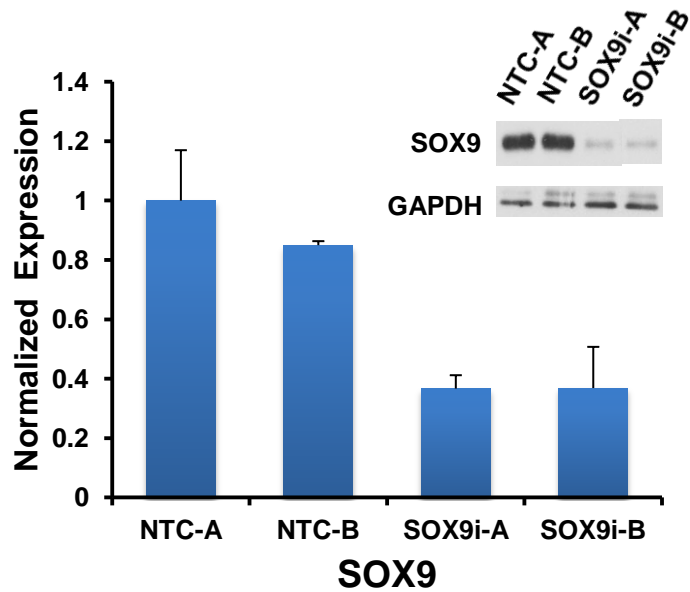


Figure S2. SOX9 knockdown in VCaP cells.

Cells were transfected with two independent siRNAs and total RNA and proteins were collected at 72 hrs post-transfection. The level of SOX9 were measured by qRT-PCR for mRNA or Western Blot for protein (insert). NTC: Non-Target Control siRNA.

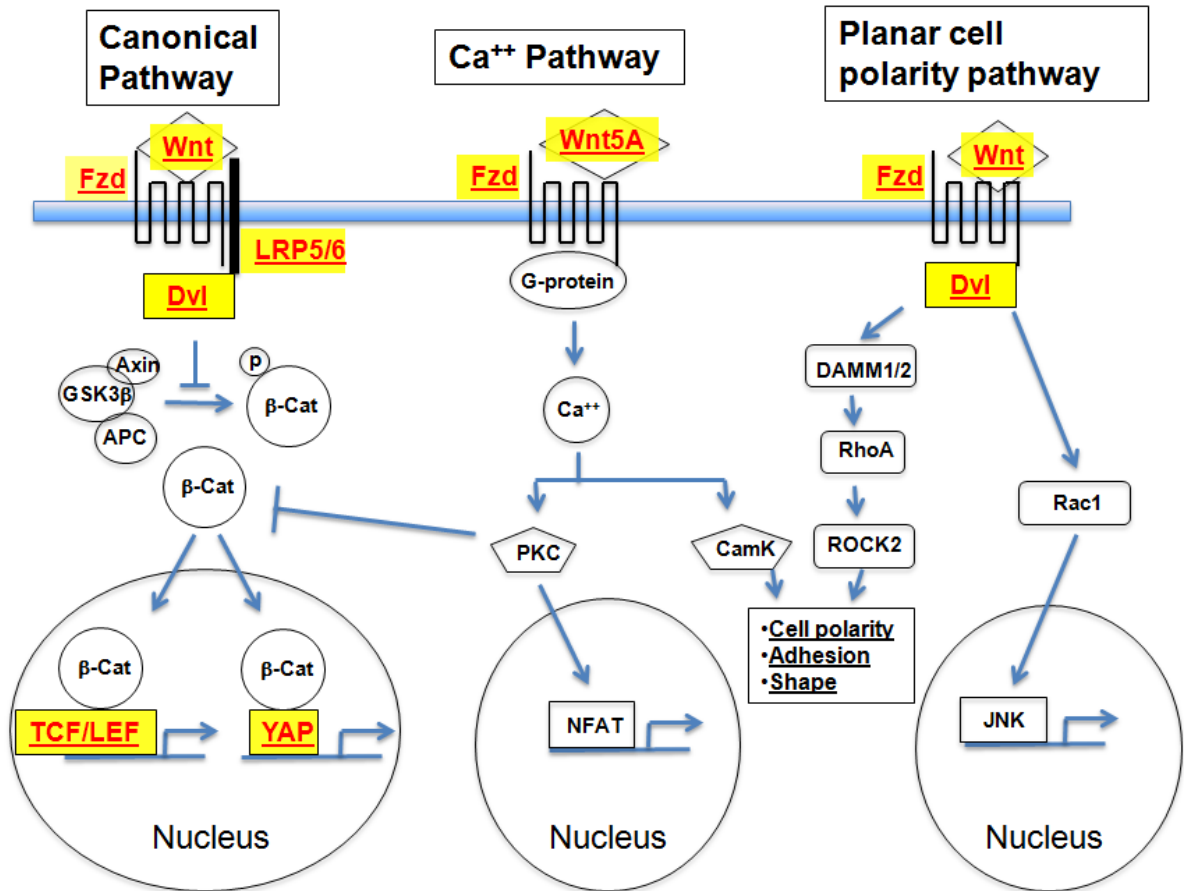
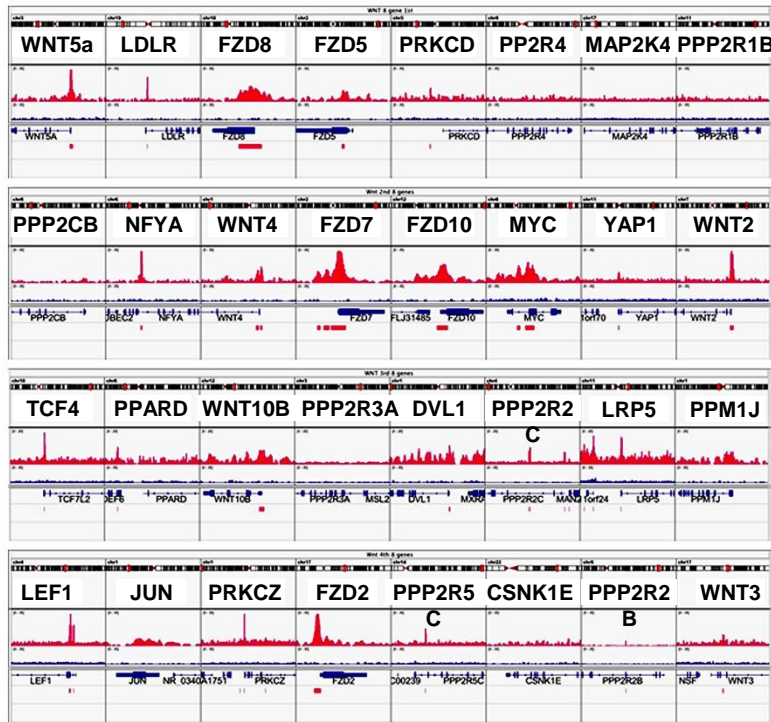


Figure S3. Schematic demonstration of the broad SOX9 effect on WNT signaling. The canonical and non-canonical WNT pathways are shown and those genes modulated by SOX9 are highlighted in yellow.

A



B

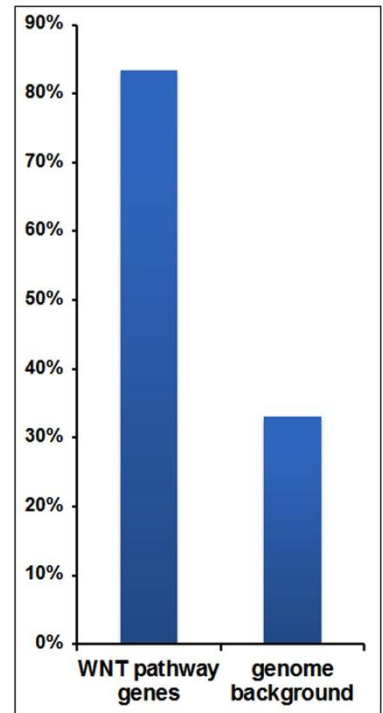


Figure S4. Enriched SOX9 binding to WNT pathway genes.

A. Representative SOX9 ChIP-seq binding profiles and peak calling of WNT component genes in VCaP cells. **B.** The percentage of genes with SOX9 binding sites within 20Kb of TSS in WNT pathways compared to the percentage of genes in human genome.

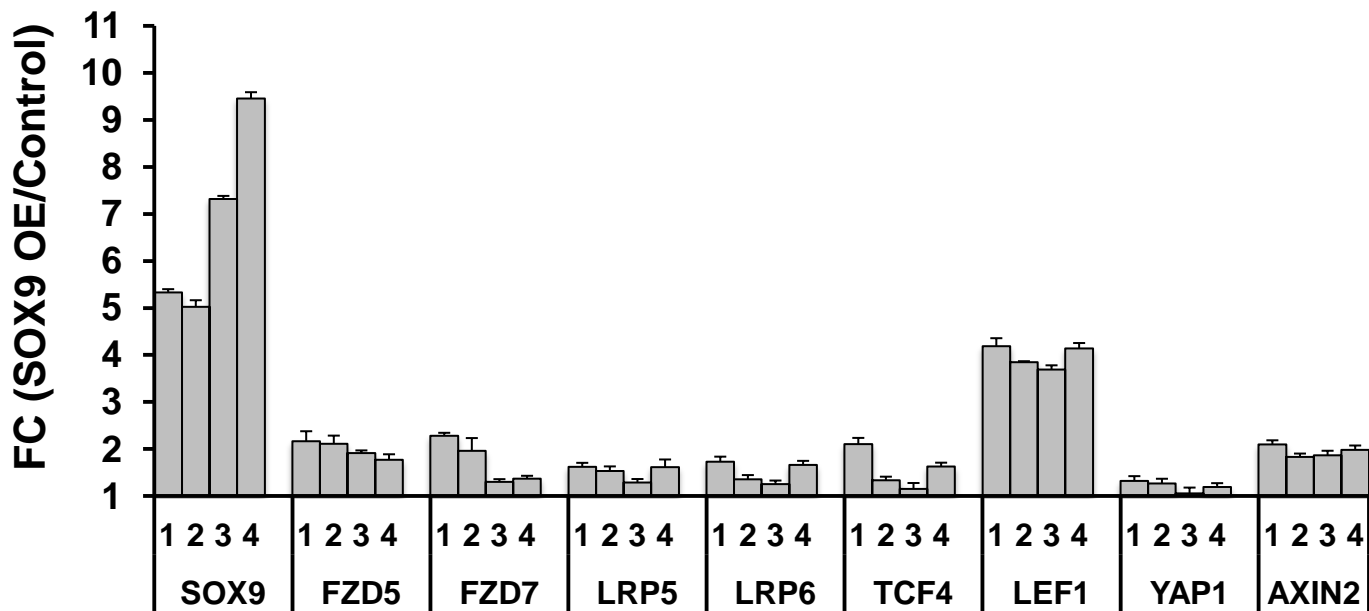


Figure S5. Upregulation of multiple WNT components in LNCaP xenografts with SOX9 over-expression.

Levels of SOX9 and its regulated genes were measured by qRT-PCR in xenografts from an induced LNCaP SOX9 overexpression cell line (SOX9 OE, n=4). The relative mRNA levels are shown as fold change (FC) of SOX9-OE xenografts compared with un-induced control. Error Bar: SD

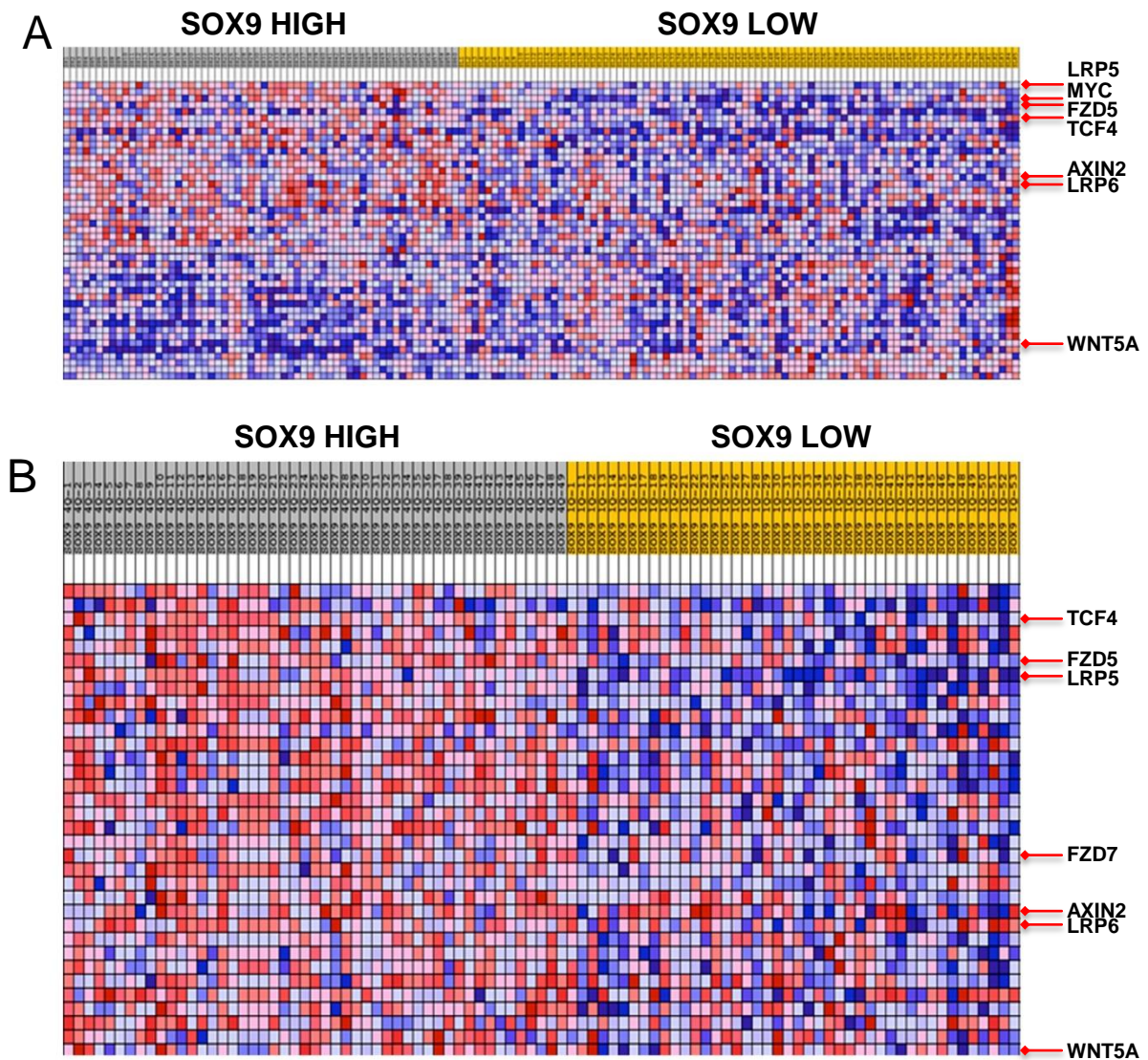


Figure S6. SOX9 associated expression of WNT signaling in clinical PCa. Heatmaps of the expression of WNT pathway components among patients with high versus low levels of SOX9 expression in the TCGA PCa dataset (**A**) or in an MSKCC PCa dataset (**B**). The arrows highlight the SOX9 regulated genes identified in VCaP cells. AXIN2 as a WNT activity readout is also highlighted.

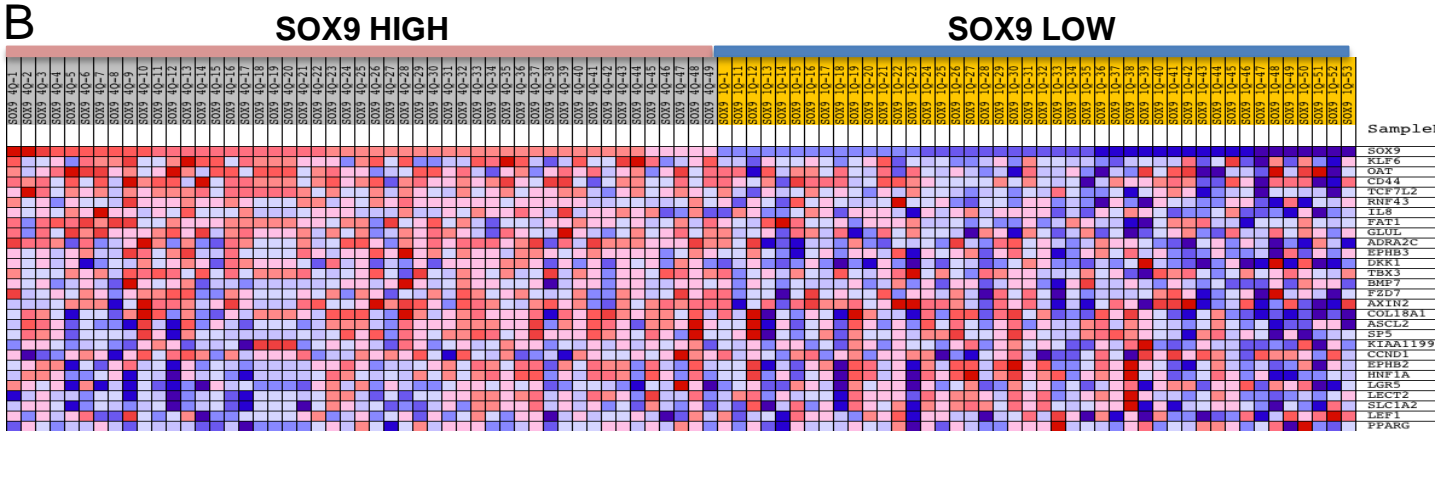
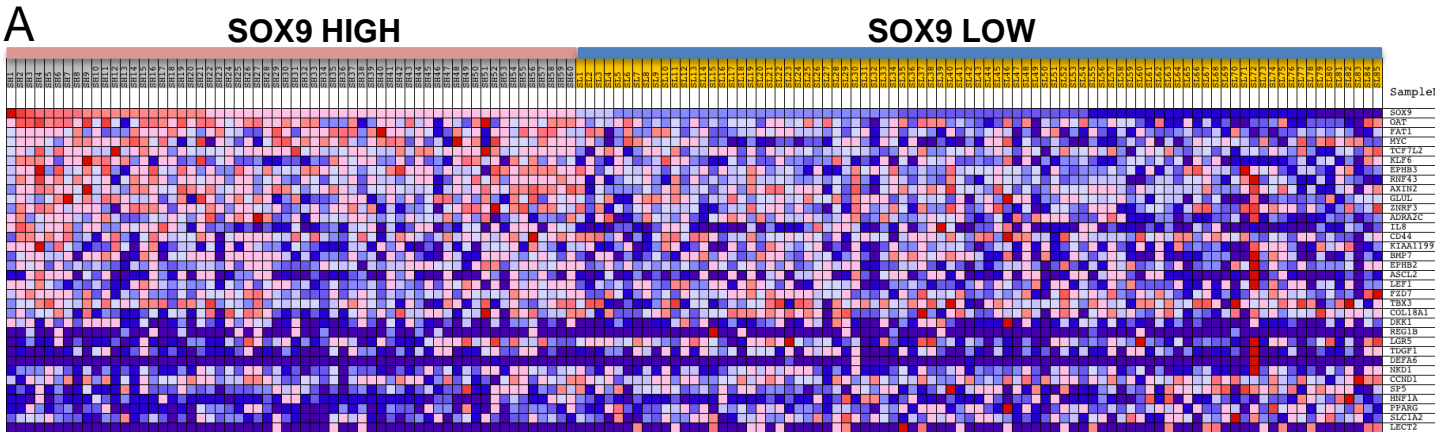


Figure S7. Enriched WNT activation signature in SOX9 high patient group. Heatmaps of the expression of WNT activation signature (adapted from reference 43) are shown to compare their expression among patients with high versus low levels of SOX9 expression in the TCGA data set **(A)** or in an MSKCC data set **(B)**. Note: TCF7L2 is an alias for TCF4.

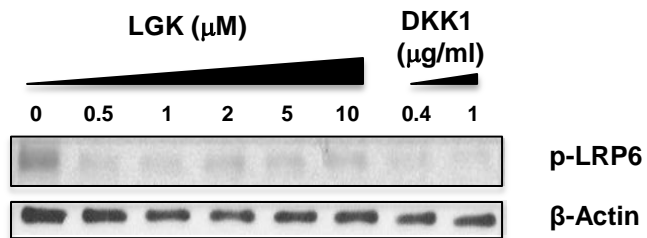


Figure S8. WNT inhibitors suppress endogenous WNT activation in VCaP cells. VCaP cells were treated with LGK974 or DKK1 at the indicated doses for 19 hours and were then lysed and immunoblotted.

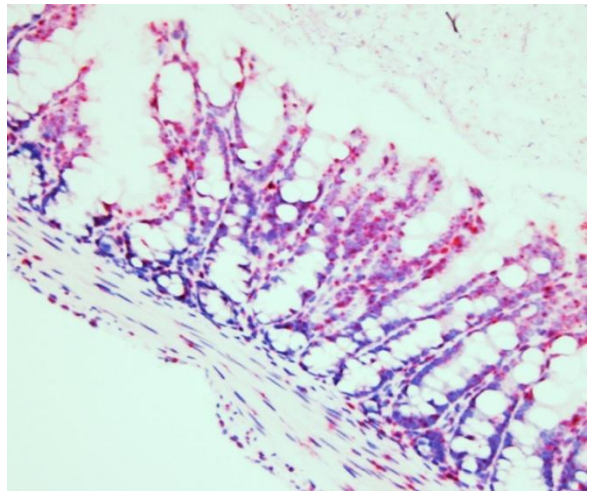
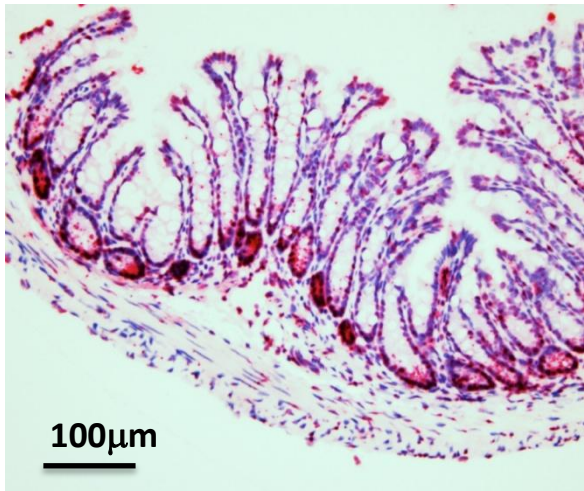
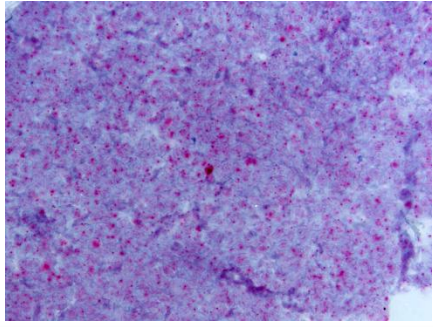
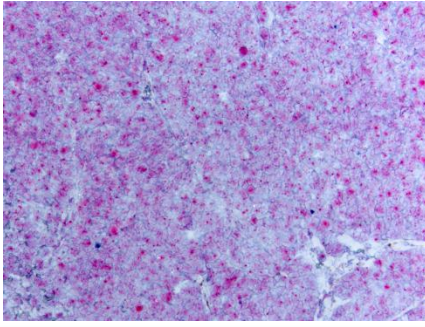
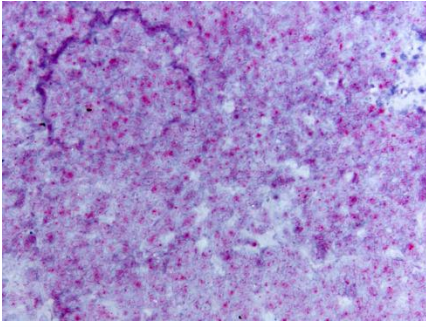
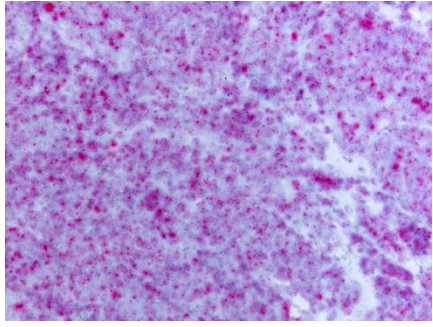
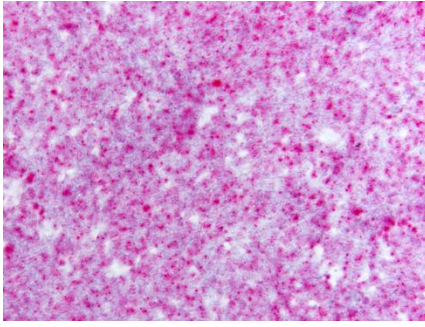
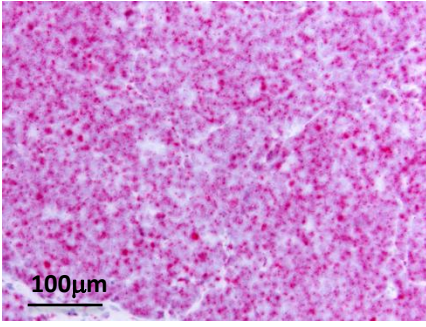
A**Control****LGK****AXIN2 (ISH)****B****Control****LGK****AXIN2 (ISH)**

Figure S9. Inhibition of WNT activity after LGK974 treatment in mice.

A. WNT activity in small intestine were measured by ISH of AXIN2 mRNA level in mice treated with carrier (control) or LGK. **B.** The spectrum of AXIN2 ISH intensities among VCaP xenografts from control or LGK treated mice. Scale bar: 100 μm.



Figure S10. Comparable SOX9 protein Levels between LNCaP-SOX9 OE and VCaP xenografts. Proteins were extracted from xenografts derived from LNCaP-SOX9 cell line without (LNCaP SOX9 Uninduce) or with (LNCaP SOX9 OE induce) doxycycline mediated SOX9 induction; from VCaP or from the parental LNCaP cell lines. SOX9 immunoblotting is shown. Vinculin blot is also shown as an internal loading control.

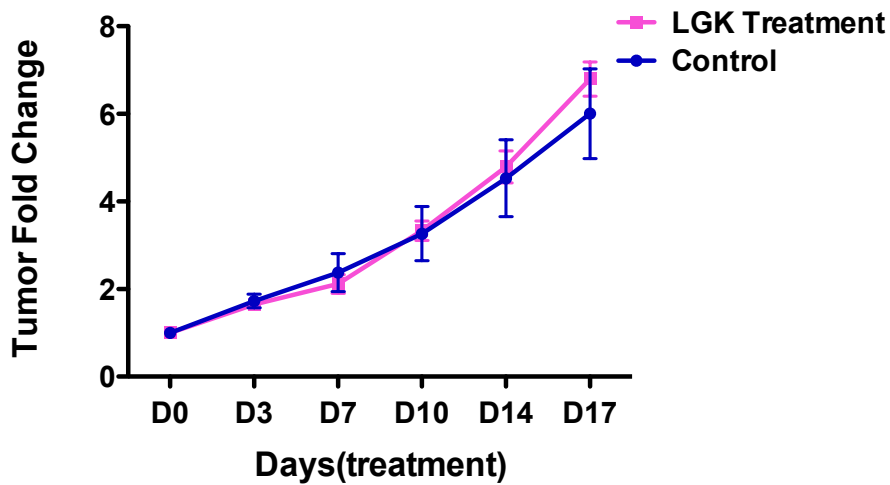


Figure S11. LGK974 does not affect growth of xenografts derived from the parental LNCaP PCa cells. Nude mice with established subcutaneous LNCaP xenografts were treated with carrier (n=8) or LGK974 (3mg/kg, daily IP injection, n=8). Tumor volume fold change was calculated by normalizing tumor volume at each time point to its Day 0 volume. Error Bar: SEM

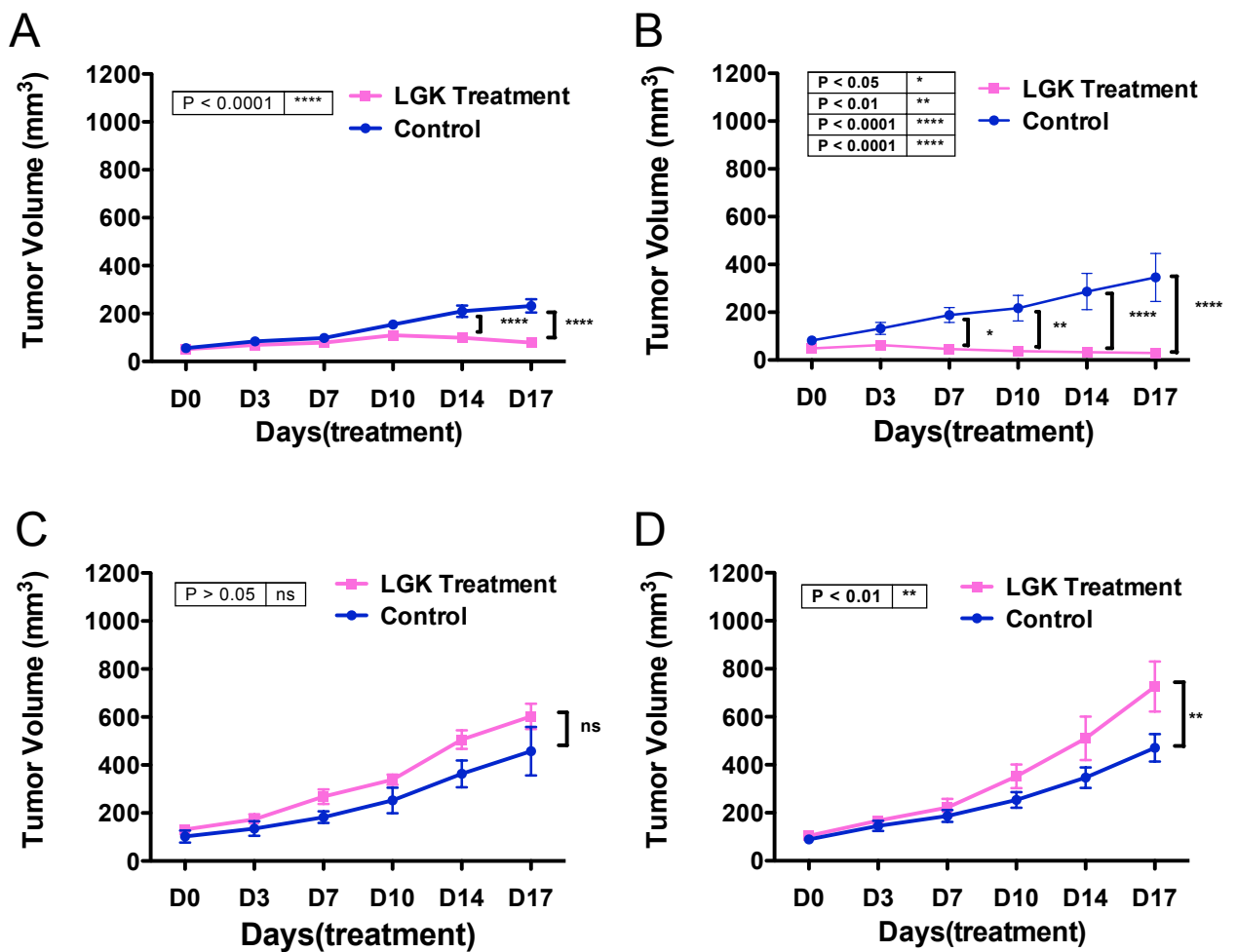


Figure S12. Inhibition of SOX9 dependent WNT activity reduces tumor growth *in vivo*. **A-D.** Mice with established subcutaneous xenografts from each group were randomly assigned to the control or LGK974 treatment subgroups and the tumor volumes were followed. Mice were treated daily with intraperitoneal injection of carrier (Control) or LGK974 (3mg/kg). **A.** SCID mice with established subcutaneous VCaP xenografts (Control, n=16; or LGK974 treated, n=16). **B-C.** SCID mice with inducible SOX9-overexpressing LNCaP cells were divided into induced (fed with doxycycline food and water) or un-induced (fed with regular food and water) groups. **B,** SOX9 induced (Control, n=8; and LGK treated, n=6). **C,** SOX9 un-induced (Control, n=4; and LGK treated, n=3). **D.** Nude mice with established subcutaneous wild type LNCaP xenografts (Control, n=8; or LGK974 treated, n=8). Two way ANOVA with Bonferroni post-tests were used to compare replicate means at each time point. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Error bar: Standard Error of Mean, SEM.

Supplemental Tables

ID	Sym	Consensus	Numhits	Mean	Cutoff	Zscore	p-value
M00189	AP-2	..CCC.C.GGCG	7544	-0.04	2.95	-15.93	1.00E-30
M00915	AP-2CC.C.GGC.	7541	-0.04	2.56	-14.86	1.00E-30
M00800	AP-2	G.C.SCRGGC.G.G..	7239	-0.04	4.12	-14.52	1.00E-30
M00469	AP-2alpha	GCC....G.	7480	-0.03	4.01	-11.72	1.00E-30
M00470	AP-2gamma	GCC...GG.	7498	-0.03	3.94	-10.35	2.01E-25
M01047	AP-2alphaA	..CGCCT.AGGCG..	7299	-0.02	1.02	-4.01	3.06E-05
M01045	AP-2alphaA	...GCCT.AGGG...	7125	0	3.18	3.1	9.99E-01
M00918	E2F	TTTGGCGC.	7126	-0.05	3.54	-19.86	1.00E-30
M00920	E2F	..CGCGCSAAA.	7280	-0.04	3.05	-16.05	1.00E-30
M00919	E2F	.CGCGCCAAA.	7404	-0.04	2.81	-14.98	1.00E-30
M00425	E2F	TTTCGCGC	2106	-0.07	5.85	-12.87	1.00E-30
M00516	E2F	TTTSGCGCG...	6784	-0.03	3.07	-10.46	6.88E-26
M00427	E2F	TTTSGCGC	4649	-0.03	5.3	-7.46	4.48E-14
M00050	E2F	TTTSGCGC	5715	-0.03	3.83	-6.99	1.38E-12
M00426	E2F	TTTSGCGC	904	-0.05	6.98	-5	2.80E-07
M01114	E2F	..TTTCCC GC.	1062	-0.03	7.48	-2.02	2.16E-02
M00024	E2F	T..GCGCGAAAA.TG	52	-0.01	9.53	2.07	9.81E-01
M00775	NF-YRCCAATCA	2048	-0.17	7.83	-36.3	1.00E-30
M00185	NF-Y	.AACCAAT.A.	2081	-0.16	7.63	-34.53	1.00E-30
M00209	NF-Y	.CTGATTGGYTA..	1004	-0.19	7.66	-27.24	1.00E-30
M00716	ZF5	G.GCGCGR	7284	-0.08	3.99	-31.16	1.00E-30
M00695	ETF	G.GGCGG	7361	-0.06	5.02	-23.14	1.00E-30
M00243	Egr-1	.TGCGTGGGCGT	7462	-0.02	0.95	-4.37	6.26E-06

Table S1. Enriched motifs of potential SOX9 cofactors in VCaP.

A list of the top ranked transcriptional factor binding motifs within 600 bp of the summits of the SOX9 binding peaks based on SOX9 Chip-seq results. ID: motif ID; Sym: transcription factor symbol; Numhits: number of hits.

Gene Symbol	FC ([SOX9i-A] vs [NTC])	FC ([SOX9i-B] vs [NTC])	Response to SOX9i
SOX9	-1.781	-2.964	down
PLAT	-7.466	-11.159	down
CCL2	-6.653	-9.334	down
ART3	-2.236	-5.747	down
NPY6R	-4.004	-5.615	down
TNFSF15	-2.762	-5.101	down
ESRRG	-4.026	-4.880	down
CXCL10	-2.718	-4.829	down
CCL20	-3.056	-4.407	down
IL8	-3.264	-4.379	down
FZD7	-2.696	-4.282	down
LOC100506548///RPL37	-1.594	-4.256	down
PTH2R	-3.628	-4.034	down
DSCR6	-1.580	-3.884	down
LTF	-2.497	-3.799	down
TESC	-2.348	-3.741	down
CA12	-1.753	-3.694	down
RBM24	-2.188	-3.581	down
CXCL5	-2.208	-3.580	down
SCUBE2	-3.211	-3.467	down
ERP27	-1.639	-3.401	down
MAB21L3	-1.715	-3.336	down
PDGFC	-4.085	-3.326	down
TXNIP	-2.277	-3.230	down
CXCL1	-2.682	-3.213	down
C17orf99	-2.046	-3.186	down
MUM1L1	-2.805	-3.162	down
LYRM7	-1.716	-3.147	down
KISS1R	-1.931	-3.013	down
NRIP3	-2.949	-2.960	down
GADD45A	-2.307	-2.899	down
ARRDC4	-2.900	-2.769	down
SLC24A2	-1.789	-2.635	down
LOC100505865	-1.904	-2.610	down
P4HA1	-2.602	-2.592	down
SLC16A10	-1.968	-2.578	down
MXI1	-2.321	-2.530	down
IL17RD	-1.753	-2.524	down
EPB41L4A-AS1	-1.554	-2.509	down
VEGFA	-1.850	-2.503	down
CXCR7	-1.833	-2.499	down
CXCL6	-1.827	-2.493	down
TGIF2	-2.801	-2.474	down
PRKX///PRKY	-1.531	-2.473	down
TIFA	-1.988	-2.447	down
RGS7	-1.621	-2.438	down
REPS2	-1.662	-2.426	down
CTTNBP2	-2.753	-2.417	down
ADORA2B///LOC100652851///LOC100653318	-1.968	-2.412	down
C17orf48	-1.552	-2.396	down
KLF10	-2.146	-2.376	down
PERP	-1.593	-2.372	down
C8orf4	-2.208	-2.368	down
SLC22A16	-1.839	-2.364	down
RNF44	-1.542	-2.364	down
IKBKB	-1.711	-2.348	down

ACSL5	-2.514	-2.331	down
LINGO1	-1.719	-2.308	down
FAM174B	-1.585	-2.299	down
HSPG2	-1.606	-2.295	down
CXCL3	-1.866	-2.285	down
PFKP	-1.789	-2.275	down
SNAP25	-1.553	-2.270	down
ARHGAP24	-2.578	-2.258	down
MXRA7	-1.943	-2.249	down
LOC100506776	-2.194	-2.240	down
YAP1	-2.036	-2.232	down
TOX3	-1.501	-2.231	down
PAQR5	-1.988	-2.221	down
GSTO2	-2.072	-2.199	down
FAM3D	-1.563	-2.197	down
MCC	-1.644	-2.184	down
ADA	-1.506	-2.150	down
SCN4B	-2.188	-2.140	down
GABBR1///UBD	-2.090	-2.139	down
PROCR	-2.092	-2.134	down
TNFRSF11A	-2.571	-2.126	down
BIRC3	-2.115	-2.126	down
PODXL	-1.846	-2.125	down
CAMKV	-1.500	-2.104	down
DNASE1L3	-1.536	-2.092	down
GGT1///GGT2///GGTLC2/// GGT3P	-1.736	-2.084	down
GGT1///GGT2///GGTLC2/// GGT3P///GGT8P	-1.724	-2.082	down
LITAF	-1.721	-2.076	down
TNFSF10	-2.151	-2.058	down
C11orf70	-2.113	-2.056	down
NRCAM	-1.880	-2.056	down
GGTLC1	-1.677	-2.051	down
CEP68	-1.556	-2.044	down
GGT1///GGT2///GGTLC2/// GGT3P///GGT8P	-1.704	-2.038	down
LOC645249	-1.876	-2.022	down
PRAME	-2.315	-2.012	down

Table S2. SOX9 activated gene in VCaP cells.

Relative fold changes (FC) of mRNA levels measured by microarray analyses. Those genes randomly selected for further qPT-PCR validation are highlighted in yellow.

Gene Symbol	FC ([SOX9i-A] vs [NTC])	FC ([SOX9i-B] vs [NTC])	Response to SOX9i
SOX9	-1.781	-2.964	down
ANXA1	1.676	4.072	up
BHLHE40	1.781	2.672	up
INSIG1	2.126	2.005	up
LUM	1.624	4.103	up
ARG2	3.510	2.448	up
S100P	2.007	2.183	up
TOX	1.664	2.139	up
IDI1	1.665	2.434	up
DPYD	1.672	2.119	up
NAP1L3	1.622	2.804	up
TFF1	1.604	2.902	up
MMP10	2.940	4.258	up
HMGCS1	1.817	2.796	up
TAF13	1.545	2.240	up
WNT5A	2.085	3.036	up
GCG	4.701	2.200	up
RORB	2.507	2.017	up
UGT2B4	1.800	9.602	up
SI	1.868	6.733	up
CSRP2	1.550	2.034	up
UGT2B17	1.672	7.479	up
SLC30A4	3.060	3.967	up
SHH	2.243	2.159	up
ID1	2.043	2.751	up
MSMO1	1.504	2.343	up
ACAT2	1.703	2.686	up
GPR37	1.822	2.842	up
ALB	2.475	2.963	up
UGT2B28	1.663	5.258	up
KCTD12	1.976	2.405	up
PVRL3	1.699	2.988	up
OAZ3	1.771	2.004	up
MIR22///MIR22H G	2.117	2.912	up
HLA-DRB1	1.722	2.007	up
OSTM1	2.116	2.861	up
SAR1B	1.613	2.170	up
GREM1	1.587	2.024	up
C7orf63	1.870	2.065	up
LRRTM4	2.447	2.100	up
COL5A2	2.220	2.411	up
ARHGEF26	2.014	2.030	up
ADAMTS1	2.144	2.400	up
REG4	3.131	2.124	up
C15orf48	2.844	3.141	up
TMEM79	1.677	2.177	up
FAM84A///LOC65 3602	1.708	2.846	up
STARD4	2.226	3.595	up
SAMD5	2.012	2.177	up
SLC45A3	2.446	2.179	up
TMEM178A	3.048	3.246	up
RUSC1-AS1	1.640	2.365	up
LOC100507025	1.663	2.595	up
DSEL	2.546	4.428	up
CDH26	2.119	2.073	up
GPR137C	1.732	2.741	up
HMG2P46	9.911	4.638	up
RFX6	3.808	3.023	up
C15orf37	1.552	2.448	up
LOC401068	1.695	2.620	up
LOC100506995	1.572	2.924	up

Table S3. SOX9 Suppressed genes in VCaP cells.

Relative fold changes (FC) of mRNA levels measured by microarray analyses. Those genes randomly selected for further qPT-PCR validation are highlighted in yellow.

GENESET NAME	SIZE	ES	NES	NOM p-val
KEGG_ALLOGRAFT_REJECTION	34	0.456	1.363	<0.001
KEGG_RIBOSOME	79	0.502	1.253	<0.001
KEGG_GRAFT_VERSUS_HOST_DISEASE	37	0.380	1.230	<0.001
KEGG_MATURITY_ONSET_DIABETES_OF_THE_YOUNG	19	0.475	1.202	<0.001
KEGG_HEDGEHOG_SIGNALING_PATHWAY	53	0.351	1.197	<0.001
KEGG_INOSITOL_PHOSPHATE_METABOLISM	46	0.321	1.179	<0.001
KEGG_BASAL_CELL_CARCINOMA	53	0.403	1.163	<0.001
KEGG_MELANOGENESIS	98	0.342	1.161	<0.001
KEGG_APOPTOSIS	82	0.306	1.157	<0.001
KEGG_PRION_DISEASES	35	0.333	1.139	<0.001
KEGG_CALCIIUM_SIGNALING_PATHWAY	171	0.222	1.128	<0.001
KEGG_TYPE_I_DIABETES_MELLITUS	40	0.409	1.126	<0.001
KEGG_MAPK_SIGNALING_PATHWAY	256	0.311	1.126	<0.001
KEGG_DILATED_CARDIOMYOPATHY	89	0.278	1.119	<0.001
KEGG_GALACTOSE_METABOLISM	25	0.355	1.096	<0.001
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	252	0.206	1.095	<0.001
KEGG_ANTIEN_PROCESSING_AND_PRESENTATION	81	0.290	1.091	<0.001
KEGG_GLYXYLATE_AND_DICARBOXYLATE_METABOLISM	15	0.419	1.091	<0.001
KEGG_NOTCH_SIGNALING_PATHWAY	41	0.277	1.087	<0.001
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	82	0.272	1.077	<0.001
KEGG_HEMATOPOIETIC_CELL_LINEAGE	83	0.242	1.073	<0.001
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	107	0.278	1.073	<0.001
KEGG_TYPE_II_DIABETES_MELLITUS	44	0.288	1.053	<0.001
KEGG_LONG_TERM_DEPRESSION	66	0.253	1.052	<0.001
KEGG_JAK_STAT_SIGNALING_PATHWAY	151	0.227	1.051	<0.001
KEGG_PENTOSE_PHOSPHATE_PATHWAY	26	0.428	1.041	<0.001
KEGG_CELL_ADHESION_MOLECULES_CAMS	125	0.307	1.038	<0.001
KEGG_GLYCOLYSIS_GLUONEOGENESIS	60	0.357	1.031	<0.001
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_KERATAN_SULFATE	15	0.450	1.030	<0.001
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	129	0.241	1.026	<0.001
KEGG_ONE_CARBON_POOL_BY_FOLATE	16	0.361	1.016	<0.001
KEGG_SMALL_CELL_LUNG_CANCER	82	0.274	1.005	<0.001
KEGG_PURINE_METABOLISM	149	0.361	1.000	<0.001
KEGG_CYSTEINE_AND_METHIONINE_METABOLISM	31	0.405	1.000	<0.001
KEGG_O_GLYCAN_BIOSYNTHESIS	29	0.526	1.000	<0.001
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_HEPARAN_SULFATE	25	0.547	1.000	<0.001
KEGG_RNA_POLYMERASE	28	0.347	1.000	<0.001
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	245	0.516	1.000	<0.001
KEGG_CHEMOKINE_SIGNALING_PATHWAY	173	0.500	1.000	<0.001
KEGG_WNT_SIGNALING_PATHWAY	144	0.352	1.000	<0.001
KEGG_DORSO_VENTRAL_AXIS_FORMATION	23	0.301	1.000	<0.001
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	66	0.439	1.000	<0.001
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	98	0.348	1.000	<0.001
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	51	0.635	1.000	<0.001
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	65	0.372	1.000	<0.001
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	51	0.343	1.000	<0.001
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	45	0.395	1.000	<0.001
KEGG_TASTE_TRANSDUCTION	44	0.494	1.000	<0.001
KEGG_THYROID_CANCER	29	0.389	1.000	<0.001
KEGG_BLADDER_CANCER	40	0.329	1.000	<0.001
KEGG_ASTHMA	27	0.512	1.000	<0.001
KEGG_AUTOIMMUNE_THYROID_DISEASE	49	0.295	1.000	<0.001
KEGG_PRIMARY_IMMUNODEFICIENCY	35	0.467	1.000	<0.001
KEGG_PATHWAYS_IN_CANCER	315	0.253	0.992	0.510
KEGG_P53_SIGNALING_PATHWAY	64	0.373	0.983	0.493
KEGG_PYRIMIDINE_METABOLISM	88	0.287	0.977	0.493
KEGG_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTIION	62	0.341	0.971	0.510

KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	34	0.303	0.971	0.510
KEGG_VIRAL_MYOCARDITIS	67	0.248	0.969	0.510
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	73	0.202	0.968	0.493
KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS	49	0.319	0.968	0.510
KEGG_CARDIAC_MUSCLE_CONTRACTION	70	0.280	0.954	0.510
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	107	0.199	0.922	0.510
KEGG_GLUTATHIONE_METABOLISM	45	0.232	0.919	0.510
KEGG_GLYCEROLIPID_METABOLISM	43	0.244	0.909	0.493
KEGG_BASE_EXCISION_REPAIR	33	0.440	0.903	0.493
KEGG_ECM_RECEPTOR_INTERACTION	81	0.240	0.896	0.510
KEGG_MISMATCH_REPAIR	22	0.524	0.891	0.493
KEGG_NUCLEOTIDE_EXCISION_REPAIR	44	0.319	0.870	0.493
KEGG_HOMOLOGOUS_RECOMBINATION	26	0.453	0.866	0.493
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GANGLIO_SERIES	15	0.280	0.860	0.510
KEGG_LEISHMANIA_INFECTION	62	0.228	0.856	0.510
KEGG_AXON_GUIDANCE	127	0.241	0.856	0.510
KEGG_OLFACTORY_TRANSDUCTION	114	0.131	0.822	0.493
KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	32	0.260	0.782	0.510
KEGG_GLYCOSAMINOGLYCAN_DEGRADATION	21	0.287	0.750	0.510
KEGG_DNA_REPLICATION	34	0.426	0.738	0.493
KEGG_NITROGEN_METABOLISM	23	0.300	0.733	0.510

Table S4. GSEA of SOX9 regulated genes in VCaP cells.

A list of enriched gene sets based on GSEA of genes differentially expressed after Non-Target-Control (NTC) vs SOX9 siRNA transfection. Enriched signaling pathways shared with those found in clinical data sets are highlighted in yellow. ES: Enrichment Score; NES: Normalized Enrichment Score; NOM p-val: Nominal p-value.

GENESET NAME	SIZE	ES	NES	NOM p-val
KEGG_NOTCH_SIGNALING_PATHWAY	45	0.678	1.762	0.002
KEGG_TASTE_TRANSDUCTION	27	0.654	1.595	0.006
KEGG_JAK_STAT_SIGNALING_PATHWAY	113	0.622	1.518	0.039
KEGG_DORSO_VENTRAL_AXIS_FORMATION	21	0.697	1.485	0.036
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	87	0.556	1.394	0.122
KEGG_ADHERENS_JUNCTION	71	0.508	1.357	0.070
KEGG_O_GLYCAN_BIOSYNTHESIS	27	0.557	1.331	0.103
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROITIN_SULFATE	22	0.582	1.315	0.148
KEGG_ABC_TRANSPORTERS	39	0.535	1.305	0.150
KEGG_SMALL_CELL_LUNG_CANCER	84	0.500	1.299	0.133
KEGG_LEISHMANIA_INFECTION	67	0.597	1.285	0.209
WNT_ACTIVATION_SIGNATURE	33	0.597	1.284	0.166
KEGG_THYROID_CANCER	29	0.501	1.283	0.132
KEGG_ETHER_LIPID_METABOLISM	26	0.501	1.277	0.133
KEGG_ACUTE_MYELOID_LEUKEMIA	57	0.476	1.241	0.173
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	198	0.534	1.240	0.243
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	54	0.492	1.228	0.212
KEGG_GRAFT_VERSUS_HOST_DISEASE	30	0.680	1.209	0.323
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	57	0.510	1.196	0.253
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	36	0.628	1.178	0.340
KEGG_LONG_TERM_POTENTIATION	63	0.421	1.167	0.230
KEGG_ALLOGRAFT_REJECTION	31	0.656	1.159	0.395
KEGG_APOPTOSIS	83	0.422	1.146	0.272
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	102	0.466	1.141	0.313
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	41	0.486	1.137	0.300
KEGG_BASAL_CELL_CARCINOMA	51	0.455	1.135	0.281
KEGG_CHRONIC_MYELOID_LEUKEMIA	72	0.399	1.125	0.261
KEGG_WNT_SIGNALING_PATHWAY	141	0.374	1.108	0.301
KEGG_N_GLYCAN_BIOSYNTHESIS	46	0.508	1.103	0.363
KEGG_GAP_JUNCTION	81	0.416	1.102	0.316
KEGG_LONG_TERM_DEPRESSION	58	0.396	1.088	0.318
KEGG_PENTOSE_PHOSPHATE_PATHWAY	25	0.464	1.080	0.383
KEGG_TIGHT_JUNCTION	117	0.364	1.077	0.305
KEGG_CYSTEINE_AND_METHIONINE_METABOLISM	28	0.446	1.061	0.388
KEGG_GNRH_SIGNALING_PATHWAY	86	0.369	1.047	0.377
KEGG_PATHWAYS_IN_CANCER	308	0.357	1.041	0.379
KEGG_AUTOIMMUNE_THYROID_DISEASE	33	0.539	1.016	0.498
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	102	0.406	1.007	0.434
KEGG_PROSTATE_CANCER	87	0.347	1.003	0.473
KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION	22	0.426	0.993	0.470
KEGG_PRION_DISEASES	29	0.417	0.993	0.460
KEGG_TYPE_I_DIABETES_MELLITUS	36	0.511	0.987	0.519
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	37	0.397	0.984	0.463
KEGG_MAPK_SIGNALING_PATHWAY	233	0.334	0.976	0.494
KEGG_PRIMARY_IMMUNODEFICIENCY	30	0.528	0.965	0.583
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	74	0.399	0.958	0.509
KEGG_CELL_ADHESION_MOLECULES_CAMS	122	0.402	0.957	0.517
KEGG_TYPE_II_DIABETES_MELLITUS	40	0.366	0.947	0.533
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	65	0.398	0.928	0.567
KEGG_BLADDER_CANCER	42	0.351	0.915	0.581
KEGG_ASTHMA	21	0.508	0.913	0.603
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	61	0.322	0.903	0.604
KEGG_SELENOAMINO_ACID_METABOLISM	26	0.372	0.900	0.586
KEGG_STARCH_AND_SUCROSE_METABOLISM	27	0.365	0.892	0.619
KEGG_AXON_GUIDANCE	125	0.320	0.889	0.610
KEGG_ENDOMETRIAL_CANCER	51	0.329	0.886	0.642

KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_HEPARAN_SULFATE	25	0.353	0.865	0.690
KEGG_ONE_CARBON_POOL_BY_FOLATE	17	0.392	0.857	0.654
KEGG_COLORECTAL_CANCER	62	0.312	0.838	0.739
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	73	0.323	0.820	0.760
KEGG_ECM_RECEPTOR_INTERACTION	80	0.335	0.785	0.747
KEGG_VIRAL_MYOCARDITIS	65	0.335	0.779	0.723
KEGG_ERBB_SIGNALING_PATHWAY	85	0.275	0.774	0.851
KEGG_OTHER_GLYCAN_DEGRADATION	15	0.393	0.770	0.765
KEGG_SPHINGOLIPID_METABOLISM	37	0.303	0.767	0.800
KEGG_P53_SIGNALING_PATHWAY	67	0.293	0.746	0.827
KEGG_TGF_BETA_SIGNALING_PATHWAY	83	0.289	0.736	0.844
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES	23	0.308	0.723	0.861
KEGG_CHEMOKINE_SIGNALING_PATHWAY	164	0.284	0.721	0.817
KEGG_LINOLEIC_ACID_METABOLISM	15	0.318	0.712	0.869
KEGG_DILATED_CARDIOMYOPATHY	78	0.319	0.707	0.813
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	58	0.332	0.706	0.792
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	41	0.328	0.675	0.835
KEGG_LYSOSOME	120	0.254	0.670	0.942
KEGG_GLYCOSAMINOGLYCAN_DEGRADATION	19	0.292	0.630	0.920
KEGG_NITROGEN_METABOLISM	17	0.263	0.600	0.981
KEGG_HEMATOPOIETIC_CELL_LINEAGE	73	0.249	0.523	0.958

Table S5. GSEA of SOX9 regulated genes in TCGA PCa data set.

A list of enriched gene sets based on GSEA of genes differentially expressed in SOX9 high vs SOX9 low patients. Enriched signaling pathways shared in VCaP cell lines and in clinical samples are highlighted in yellow. WNT activation signature (excluding the SOX9 gene) was adapted from (43) and is highlighted in red. ES: Enrichment Score; NES: Normalized Enrichment Score; NOM p-val: Nominal p-value.

GENESET NAME	SIZE	ES	NES	NOM p-val
KEGG_NOTCH_SIGNALING_PATHWAY	34	0.638	1.938	<0.001
KEGG_GNRH_SIGNALING_PATHWAY	88	0.447	1.686	0.006
KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION	39	0.569	1.683	0.002
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	42	0.641	1.638	0.032
KEGG_MAPK_SIGNALING_PATHWAY	221	0.414	1.619	0.020
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROITIN_SULFATE	17	0.615	1.600	0.049
WNT_ACTIVATION_SIGNATURE	28	0.651	1.597	0.025
KEGG_GAP_JUNCTION	69	0.473	1.574	0.048
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	37	0.692	1.543	0.035
KEGG_LEISHMANIA_INFECTION	54	0.652	1.542	0.080
KEGG_FRUCTOSE_AND_MANNANOSE_METABOLISM	28	0.540	1.522	0.083
KEGG_OTHER_GLYCAN_DEGRADATION	14	0.608	1.509	0.078
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	48	0.503	1.508	0.076
KEGG_GRAFT_VERSUS_HOST_DISEASE	23	0.742	1.492	0.064
KEGG_DORSO_VENTRAL_AXIS_FORMATION	17	0.595	1.491	0.093
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	38	0.464	1.479	0.085
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_HEPARAN_SULFATE	18	0.479	1.477	0.071
KEGG_PATHWAYS_IN_CANCER	272	0.386	1.452	0.058
KEGG_TAURINE_AND_HYPOTAURINE_METABOLISM	9	0.635	1.432	0.070
KEGG_BLADDER_CANCER	35	0.469	1.419	0.054
KEGG_ALLOGRAFT_REJECTION	26	0.656	1.413	0.119
KEGG_ACUTE_MYELOID_LEUKEMIA	47	0.454	1.382	0.096
KEGG_ENDOCYTOSIS	150	0.397	1.378	0.102
KEGG_GLYCOSAMINOGLYCAN_DEGRADATION	13	0.482	1.359	0.171
KEGG_TYPE_I_DIABETES_MELLITUS	28	0.609	1.351	0.161
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	79	0.429	1.347	0.148
KEGG_VIBRIO_CHOLERAE_INFECTION	39	0.431	1.344	0.127
KEGG_INSULIN_SIGNALING_PATHWAY	114	0.376	1.342	0.099
KEGG_AUTOIMMUNE_THYROID_DISEASE	30	0.597	1.338	0.189
KEGG_CIRCADIAN_RHYTHM_MAMMAL	10	0.680	1.338	0.154
KEGG_BASAL_CELL_CARCINOMA	49	0.427	1.318	0.180
KEGG_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTI	46	0.442	1.309	0.129
KEGG_VIRAL_MYOCARDITIS	46	0.528	1.308	0.225
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	77	0.400	1.300	0.175
KEGG_VEGF_SIGNALING_PATHWAY	65	0.354	1.298	0.125
KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS	44	0.322	1.298	0.106
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	58	0.561	1.293	0.186
KEGG_PROSTATE_CANCER	68	0.442	1.291	0.103
KEGG_ABC_TRANSPORTERS	36	0.402	1.290	0.124
KEGG_ADHERENS_JUNCTION	59	0.464	1.287	0.196
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION	97	0.420	1.283	0.204
KEGG_APOPTOSIS	73	0.356	1.275	0.158
KEGG_JAK_STAT_SIGNALING_PATHWAY	122	0.371	1.268	0.154
KEGG_LYSOSOME	92	0.450	1.267	0.200
KEGG_TYPE_II_DIABETES_MELLITUS	41	0.370	1.257	0.112
KEGG_LONG_TERM_DEPRESSION	56	0.392	1.255	0.166
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	89	0.360	1.251	0.202
KEGG_ASTHMA	21	0.627	1.244	0.232
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	61	0.379	1.237	0.203
KEGG_CELL_ADHESION_MOLECULES_CAMS	106	0.448	1.233	0.245
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	68	0.465	1.227	0.250
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	59	0.366	1.219	0.190
KEGG_ENDOMETRIAL_CANCER	41	0.398	1.216	0.178
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	56	0.316	1.216	0.193
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	61	0.336	1.203	0.209
KEGG_PANTOTHENATE_AND_COA_BIOSYNTHESIS	12	0.460	1.193	0.244

KEGG_N_GLYCAN_BIOSYNTHESIS	37	0.435	1.185	0.300
KEGG_O_GLYCAN_BIOSYNTHESIS	27	0.386	1.180	0.222
KEGG_CHRONIC_MYELOID_LEUKEMIA	59	0.412	1.172	0.265
KEGG_AXON_GUIDANCE	103	0.330	1.172	0.238
KEGG_ETHER_LIPID_METABOLISM	27	0.387	1.169	0.243
KEGG_SMALL_CELL_LUNG_CANCER	69	0.383	1.161	0.278
KEGG_RIBOFLAVIN_METABOLISM	11	0.480	1.153	0.286
KEGG_ECM_RECEPTOR_INTERACTION	68	0.475	1.144	0.346
KEGG_SULFUR_METABOLISM	9	0.447	1.139	0.289
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	38	0.392	1.126	0.324
KEGG_MELANOGENESIS	87	0.298	1.125	0.264
KEGG_PRION_DISEASES	32	0.405	1.122	0.305
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	170	0.328	1.121	0.324
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	91	0.375	1.119	0.319
KEGG_THYROID_CANCER	25	0.385	1.116	0.326
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	31	0.398	1.115	0.333
KEGG_ARACHIDONIC_ACID_METABOLISM	46	0.393	1.109	0.310
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	205	0.388	1.090	0.389
KEGG_TIGHT_JUNCTION	99	0.318	1.088	0.340
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	37	0.383	1.069	0.351
KEGG_CHEMOKINE_SIGNALING_PATHWAY	144	0.301	1.064	0.365
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	84	0.307	1.056	0.389
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	106	0.291	1.053	0.395
KEGG_ALPHA_LINOLENIC_ACID_METABOLISM	17	0.416	1.052	0.364
KEGG_COLORECTAL_CANCER	53	0.378	1.050	0.424
KEGG_WNT_SIGNALING_PATHWAY	119	0.271	1.049	0.371
KEGG_DILATED_CARDIOMYOPATHY	82	0.406	1.035	0.407
KEGG_TGF_BETA_SIGNALING_PATHWAY	69	0.369	1.034	0.405
KEGG_FC_EPSILON_RI_SIGNALING_PATHWAY	67	0.272	1.029	0.373
KEGG_HEMATOPOIETIC_CELL_LINEAGE	71	0.412	1.022	0.448
KEGG_NON_SMALL_CELL_LUNG_CANCER	44	0.307	1.020	0.435
KEGG_ERBB_SIGNALING_PATHWAY	73	0.302	1.019	0.433
KEGG_PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	21	0.384	1.015	0.442
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	97	0.358	1.008	0.436
KEGG_FOCAL_ADHESION	165	0.363	0.996	0.470
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	74	0.402	0.994	0.439
KEGG_MELANOMA	53	0.272	0.993	0.449
KEGG_GLYCEROPHOSPHOLIPID_METABOLISM	61	0.257	0.985	0.484
KEGG_PURINE_METABOLISM	130	0.256	0.962	0.530
KEGG_STARCH_AND_SUCROSE_METABOLISM	25	0.360	0.959	0.509
KEGG_PANCREATIC_CANCER	62	0.326	0.949	0.532
KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	31	0.318	0.946	0.529
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GLOBO_SERIES	12	0.363	0.940	0.555
KEGG_GLYCEROLIPID_METABOLISM	36	0.276	0.938	0.575
KEGG_TASTE_TRANSDUCTION	42	0.351	0.926	0.557
KEGG_ALZHEIMERS_DISEASE	129	0.267	0.917	0.543
KEGG_CALCIIUM_SIGNALING_PATHWAY	153	0.271	0.900	0.636
KEGG_LONG_TERM_POTENTIATION	59	0.245	0.898	0.588
KEGG_GALACTOSE_METABOLISM	21	0.308	0.887	0.632
KEGG_ASCORBATE_AND_ALDARATE_METABOLISM	7	0.478	0.871	0.652
KEGG_P53_SIGNALING_PATHWAY	54	0.318	0.868	0.648
KEGG_INOSITOL_PHOSPHATE_METABOLISM	46	0.249	0.859	0.699
KEGG_PHENYLALANINE_METABOLISM	14	0.386	0.856	0.655
KEGG_ARGININE_AND_PROLINE_METABOLISM	46	0.282	0.849	0.675
KEGG_RNA_POLYMERASE	20	0.378	0.846	0.633
KEGG_PRIMARY_IMMUNODEFICIENCY	31	0.328	0.827	0.664
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	33	0.295	0.814	0.744
KEGG_TYROSINE_METABOLISM	33	0.282	0.814	0.713
KEGG_CARDIAC_MUSCLE_CONTRACTION	62	0.242	0.787	0.824
KEGG_RENAL_CELL_CARCINOMA	59	0.265	0.755	0.782

KEGG_MATURITY_ONSET_DIABETES_OF_THE_YOUNG	20	0.336	0.744	0.812
KEGG_GLYCOLYSIS_GLUONEOGENESIS	54	0.232	0.738	0.824
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES	23	0.243	0.728	0.874
KEGG_PENTOSE_PHOSPHATE_PATHWAY	22	0.292	0.703	0.767
KEGG_HUNTINGTONS_DISEASE	139	0.226	0.688	0.779
KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION	19	0.244	0.660	0.954
KEGG_MTOR_SIGNALING_PATHWAY	46	0.233	0.651	0.956
KEGG_PENTOSE_AND_GLUCURONATE_INTERCONVERSIONS	7	0.332	0.650	0.878
KEGG_STEROID_HORMONE_BIOSYNTHESIS	23	0.238	0.622	0.963
KEGG_NICOTINATE_AND_NICOTINAMIDE_METABOLISM	21	0.236	0.610	0.966
KEGG_SPLICEOSOME	94	0.217	0.494	0.879

Table S6. GSEA of SOX9 regulated genes in a MSKCC PCa data set.

A list of enriched gene sets based on GSEA of genes differentially expressed in SOX9 high vs SOX9 low patients. Enriched signaling pathways shared in VCaP cell lines and in clinical samples are highlighted in yellow. WNT activation signature (excluding the SOX9 gene) was adapted from (43) and is highlighted in red. ES: Enrichment Score; NES: Normalized Enrichment Score; NOM p-val: Normalized p-value.

Gene Symbol	FC ([SOX9i-A] vs [NTC])	FC ([SOX9i-B] vs [NTC])	Response to SOX9i	SOX9 binding Site
FZD7	-2.705366	-4.3086505	down	Promoter
FZD10	-1.1041493	-2.5123212	down	Promoter
MYC	-1.299235	-2.4288185	down	Promoter
YAP1	-2.0436602	-2.2455935	down	Promoter
WNT2	-1.0057149	-2.00734	down	Promoter
TCF7L2	-1.6585635	-1.7348635	down	Promoter
PPARD	-1.0776321	-1.5920618	down	Adjacent Upstream
WNT10B	-1.4100173	-1.5008799	down	Promoter
PPP2R3A	-1.3735853	-1.4141906	down	No binding within 100Kb Up and Downstream
DVL1	-1.2100639	-1.4027503	down	Promoter
PPP2R2C	-1.1863034	-1.329133	down	Promoter& Intron
LRP5	-1.1017319	-1.3281375	down	Promoter
PPM1J	-1.3298713	-1.3213532	down	Promoter
LEF1	-1.1778091	-1.2759897	down	Promoter
JUN	-1.287625	-1.272834	down	Promoter
PRKCZ	-1.2250994	-1.2629664	down	Promoter
FZD2	-1.7199576	-1.237837	down	Promoter
PPP2R5C	-1.0531132	-1.2257257	down	Intron1
CSNK1E	-1.1666708	-1.2198046	down	Adjacent Downstream
PPP2R2B	-1.1469198	-1.2157998	down	Intron
WNT3	-1.1431704	-1.1987146	down	Intron
WNT5A	2.0773335	3.0172555	up	Promoter
LDLR	1.2672231	1.9285403	up	Promoter
FZD8	1.2921532	1.8435903	up	Promoter
FZD5	1.2987889	1.8212223	up	Promoter
PRKCD	1.0953419	1.4727684	up	4.9K Upstream
PPP2R4	1.1606416	1.31531	up	30K Upstream and 14K Downstream
MAP2K4	1.2701262	1.2752674	up	No binding within 100Kb Up and Downstream
PPP2R1B	1.7012435	1.2437013	up	No binding within 100Kb Up and Downstream
PPP2CB	1.076358	1.2392744	up	104Kb Upstream
NFYA	1.1331067	1.2187467	up	Intron
WNT4	1.005561	1.1887566	up	Promoter

Table S7. SOX9 regulated WNT pathway genes in VCaP cells. WNT pathway genes with absolute fold change (SOX9i-B/NTC) of more than 1.2 are listed, together with the corresponding SOX9 binding information based on SOX9 CHIP-seq results.

Supplementary Methods

RNA interference. siGENOME siRNA (GE Healthcare Dharmacon) were the following:

SOX9i-A: D-021507-02

SOX9i-B: D-021507-03

NTC-A: D-001210-02

NTC-B: D-001210-04

Quantitative reverse transcription PCR(qRT-PCR). Primers were from primer bank

(<http://pga.mgh.harvard.edu/primerbank/>), or designed using primer 3

(<http://bioinfo.ut.ee/primer3/>) and synthesized by Integrated DNA Technologies.

	Reverse Sequence	Forward Sequence
SOX9	CCTGCCCGTTCTTCACCGAC	GCTCTGGAGACTTCTGAACGAGAGC
PLAT-1	CTTCCCAGCAAATCCTTCGGG	AGCGAGCCAAGGTGTTTCAA
PLAT-2	GGTAGGCTGACCCATTCCC	AAACCCAGATCGAGACTCAAAGC
CCL2	TGGAATCCTGAACCCACTTCT	CAGCCAGATGCAATCAATGCC
CXCL10	TGATGGCCTTCGATTCTGGATT	GTGGCATTCAAGGAGTACCTC
CCL20	CGCACACAGACAACCTTTTCTTT	TGCTGTACCAAGAGTTTGCTC
IL8	AACCCTCTGCACCCAGTTTTTC	ACTGAGAGTGATTGAGAGTGGAC
CXCL5	TGGCGAACACTTGCAGATTAC	AGCTGCGTTGCGTTTGTTTAC
PDGFC-1	CTTGGGCTGTGAATACTTCCATT	GA CTCAGGCGGAATCCAACC
PDGFC-2	GGGTCTTCAAGCCCAAATCTT	ATTCACAGCCCAAGGTTTCCT
TXNIP	GCAGGTA CTCCGAAGTCTGT	TGTGTGAAGTTACTCGTGCAAA
CXCL1-1	GCCCATTCTTGAGTGTGGCTATGAC	CAGCAGGAGCGTCCGTGGC
CXCL1-2	CAGTTGGATTTGTCACTGTT CAGCATC	TGCGCCCAAACCGAAGTCATAG
GADD45A-1	CACAACACCACGTTATCGGG	GAGAGCAGAAGACCGAAAAGGA
GADD45A-2	GATCCATGTAGCGACTTTCCC	CCCTGATCCAGGCGTTTTG
KLF10-1	ACGAATCACACTTGTTGCCTG	ACTGCCAAACCTCACATTGC
KLF10-2	CAGCCTCAACATTTAGGTGGG	GCAACAAGTGTGATTGTCATAC
ARRDC4	ATTGGCGACCATGTGTCGAAT	GTTCTCTCGTCTGATTGTTCC
VEGFA	AGGGTCTCGATTGGATGGCA	AGGGCAGAATCATCACGAAGT
CXCL3	GCTCCCC TTGTT CAGTATCTTTT	CGCCCAAACCGAAGTCATAG
SHH-1	ATCGCTCGGAGTTTCTGGAGA	CTCGCTGCTGGTATGCTCG

SHH-2	GGCCCTCGTAGTGCAGAGA	GAAACTCCGAGCGATTTAAGGA
FZD7-1	AGGTGAGAACGGTAAAGAGCG	GTGCCAACGGCCTGATGTA
FZD7-2	ACGATCATGGTCATCAGGTA	CAGACGTGCAAGAGCTATGC
TCF4-1	GATCCGTTGGGGAGGTAGG	TGGAGGGCTCTTTAAGGGG
TCF4-2	GCCAGGCGATAGTGGGTAAT	GCCTCTTATCACGTACAGCAAT
YAP1-1	GCAGAGAAGCTGGAGAGGAATGAGC	AATCCCACTCCCGACAGGCC
YAP1-2	GTTTCATGGCAAACGAGGGTCAAG	ATGAACTCGGCTTCAGGTCCTCTTC
LEF1	GGCATCATTATGTACCCGGAAT	AGAACACCCCGATGACGGA
LRP6	ATGGCTTCTTCGCTGACATCA	ACGATTGTAGTTGGAGGCTTG
WNT5A	CGCCTTCTCCGATGTACTGC	ATTCTTGGTGGTCGCTAGGTA
FZD5	ACGCACAGGTAGCAGGCTGACAG	TCGGTGCTGTGCTTCATCTCCAC
LRP5	CAGGCATGGATGGAGCGGGTC	GGCTCGTTCGGCAGAAGGTG
AXIN2	TGCTGTTGTTCTCAATGTACCTT	ACCAAACTTTACGAGTAGCCAA
GAPDH	CGCCCCACTTGATTTTGA	TTGCCATCAATGACCCCTTCA

Primary antibodies used in immunoblotting

Antigen	Vendor	Catalogue number	Dilution
active β -catenin	Millipore	05-665	1:1000
SOX9	Millipore	AB-5535	1:10000
Myc	Epitomics	1472-1	1:10000
Cyclin D1	Santa Cruz	Sc-8396	1:1000
LEF1	Cell Signaling	2230	1:1000
GAPDH	Abcam	Ab-9485	1:10000
Phospho-LRP6(Ser1490)	Cell Signaling	2568	1:200

Primary antibodies used in IHC

Antigen	Vendor	Catalogue number	Dilution
YAP1	Cell Signaling	4912	1:2000
SOX9	Millipore	AB-5535	1:5000

Primary antibodies used in ChIP and ChIP-Seq

Antigen	Vendor	Catalogue number
SOX9 antibody 1 (Ab1)	Millipore	AB-5535
SOX9 antibody 1 (Ab2)	Abcam	ab3697

The primers for ChIP of Col2a1 intron1 site are the following:

Forward: 5'-TCCGCGAGGAACCAGTTTAA-3',

Reverse: 5'-TGTTTGCATTGGGAGATTGG-3'.

ISH:

The probe sets were purchased from Advanced Cell Diagnostics (ACD) and are listed as the following:

AXIN2 (Human): 400241
FZD5 (Human): 414051
FZD7 (Human): 414061
Sox9 (Mouse): 401051
Axin2 (Mouse): 400331